The Long and the Short of Mate Attraction in a Psylloid: do Semiochemicals Mediate Mating in *Aacanthocnema dobsoni* Froggatt?

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Abstract

Mating is preceded by a series of interdependent events that can be broadly categorized into searching and courtship. Long-range signals convey species- and sex-specific information during searching, while short-range signals provide information specific to individuals during courtship. Studies have shown that cuticular hydrocarbons (CHCs) can be used for mate recognition in addition to protecting insects from desiccation. In Psylloidea, four species rely on semiochemicals for long-range mate attraction. Psyllid mating research has focused on long-range mate attraction and has largely ignored the potential use of cuticular hydrocarbons (CHCs) as mate recognition cues. This study investigated whether CHCs of *Aacanthocnema dobsoni* have semiochemical activity for long- and short-range communication prior to mating. Using a solid sampler for solvent-less injection of whole psyllids into coupled gas

chromatography/mass spectrometry, we found quantitative, sex- and age-related differences in CHC profiles. Males had higher proportions of 2-MeC_{28}, 11,15-diMeC_{29}, and *n*-C_{33} alkanes, while females had higher proportions of 5-MeC_{27}, 3-MeC_{27}, 5,15-diMeC_{27}, *n*-C_{29} and *n*-C_{30} alkanes. In males and females, 84 and 68 % of CHCs varied with age, respectively. Y-tube olfactometer bioassays provided no evidence that males or females responded to odors emanating from groups of conspecifics of the opposite sex. Tests of male and female psyllids for attraction to branchlets previously occupied by conspecifics showed no evidence of attraction to possible semiochemical residues. Our short-range chemoreception bioassay showed that males were as indifferent to freshly killed individuals of either sex with intact CHC profiles as to those treated with hexane (to remove CHCs). *Aacanthocnema dobsoni* utilizes substrate–borne vibrations (SBVs) for communication. Therefore, our results indicate that SBVs are probably more important than semiochemicals for long-range mate attraction. Furthermore, CHCs are unlikely to mediate short-range mate recognition or provide mate assessment cues.

Keywords

Olfaction
Contact chemoreception
3-methylheptacosane
Psylloidea
Hemiptera Triozidae

Electronic supplementary material

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Introduction

Mating in animals is preceded by a series of events that can be broadly categorized into searching and courtship (Shuker and Simmons 2014). Mating success usually results from timely and accurate information exchange and coordination between potential mating pairs. While searching typically is mediated by long-range mate attraction signals

(which provide species- and sex-specific information), short-range signals may be utilized during courtship and may provide information specific to an individual's quality (Johansson and Jones 2007; Ringo 1996). Insects also can utilize chemical, visual, and acoustic modalities to exchange information and coordinate complex courtship behaviors (Robinson et al. 2005; Sanborn 2008; Sweeney et al. 2003). Signals can be subjected to environmental distortions and, in some cases, less desirable signalers may send deceitful signals (Bradbury and Vehrencamp 2011a). To increase signal honesty, many insect taxa rely on more than one signal modality (Elias et al. 2010). Nevertheless, there are several examples within Insecta in which information exchange is mediated *via* a single signal modality (Sueur and Aubin 2004). In such systems, critical signal characteristics may vary between searching and courtship to provide essential information at both stages (Sueur and Aubin 2004).

Insect signaling systems are highly diverse and most likely reflect phylogenetic traits retained over evolutionary time shaped by physical constraints, most notably from the environment (Bradbury and Vehrencamp 2011a; Johansson and Jones 2007). For these reasons, cuticular hydrocarbons (CHCs) and substrate–borne vibrations (SBVs) have been used in systematic studies of a range of taxa (Bagnères and Wicker-Thomas 2010; Kather and Martin 2012; Percy et al. 2006). Cuticular hydrocarbons can provide evolutionary insight into taxa because they are species-specific, with some compounds conserved among related taxa (Kather and Martin 2012; Martin et al. 2008a, b; Martin and Drijfhout 2009). Insect CHCs typically comprise a complex mixture of long-chain aliphatic and methyl-branched alkanes and alkenes (Howard and Blomquist 2005). Although their ancestral, and probable primary function, is to protect insects from desiccation (King and Sinclair 2015), studies have revealed that they can be used for communication in both social and non-social insects. In social insects, such as ants, wasps, and termites, a blend of CHCs comprises essential compounds that serve as nest mate recognition cues (Guarino et al. 2008; Howard and Blomquist 2005; Van Zweden and d'Ettorre 2010). Cuticular hydrocarbons also are utilized by some social insects to mediate dominance and fertility (Liebig 2010). In non-social insects, CHCs play a role as short-range mate recognition cues (Drijfhout et al. 2003; Steiger et al. 2013).

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The superfamily Psylloidea is highly diverse, comprising over 3800 described species distributed worldwide in all major zoogeographical regions (Austin et al. 2004; Burckhardt et al. 2014). Psyllids are exclusively phytophagous, and some species are economically important because they vector plant pathogens (Hall et al. 2013; Hung et al. 2004; Munyaneza et al. 2007). Males of four psyllid species, *Cacopsylla bidens*, *C. pyricola*, *Bactericera cockerelli*, and *Diaphorina citri*, have been shown to be attracted to "pheromones" produced by conspecific females (Guédot et al. 2009a, 2010; Horton et al. 2008; Wenninger et al. 2008). Conversely, the behavior of females of all but *B. cockerelli* is not modified by male odors (Guédot et al. 2011). Male and female *C. pyricola* are repelled by "pheromones" of the same sex, while *B. cockerelli* males are attracted to male-produced odors (Guédot et al. 2009b, 2010). Results from these studies suggest that females of these species produce "pheromones" attractive to conspecific males.

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Two compounds, 13-methylheptacosane and dodecanoic acid, have been identified as "long–range" sex attractants in *C. pyricola* and *D. citri*, respectively (Guédot et al. 2009b; Mann et al. 2012). Given a putative upper molecular weight (MW) limit for airborne sex pheromones of about 300, 13-methylheptacosane (MW = 395) is, at best, barely volatile and likely to function at short-range only (Bradbury and Vehrencamp 2011b; Lubanga et al. 2014). Although dodecanoic acid (MW = 200) is sufficiently light to act at long-range, it failed to attract *D. citri* males under field conditions (Mann et al. 2012). Dodecanoic acid has a melting point of 44–46 °C and a boiling point of 225 °C at 100 mmHg (Pence and Williams 2010). Therefore, this compound is not expected to be volatile under field conditions, which could explain its failure to attract *D. citri* males in the field. It follows, then, that semiochemicals effective for long-range mate attraction in Psylloidea are unidentified. Additionally, earlier studies have shown that males of *C. pyricola* are more attracted to pear shoots previously occupied by females compared to shoots previously unoccupied or occupied by males (Brown et al. 2009). Although this finding suggested that females left cues on host plants that were utilized by males, no further studies were conducted to investigate the existence and role of such cues in other Psylloidea species. Additionally, the possibility that CHCs may be utilized as short-range mate assessment cues has yet to

be studied.

The aim of this study was to investigate whether semiochemicals mediate long-range mate attraction and short-range mate recognition in *Aacanthocnema dobsoni* (Hemiptera: Triozidae). First, we tested whether there were any sex, mating status, and age-related differences in the CHC profiles of *A. dobsoni*. Second, we tested whether CHCs mediate long-range mate attraction and short-range mate recognition. Last, we investigated whether males and females leave chemical cues on host plant branchlets that can be utilized by one sex to locate the other.

Methods and Materials

Study Species *Aacanthocnema dobsoni* is endemic to Australia and is considered monophagous on drooping she-oak, *Allocasuarina verticillata* (Lam.) L. Johnson (Casuarinaceae) (Taylor et al. 2011). This species has been recorded only in the Australian Capital Territory, South Australia, and Tasmania; however, it is likely that it occurs throughout the entire range of *A. verticillata* in southeastern Australia. Adults can reach very high population densities in summer (*e.g.*, 50 adults/sweep, unpubl. data), and feeding by large numbers of nymphs causes chlorosis of branchlets (Steinbauer et al. unpublished data). Females usually oviposit between scale leaves, and the resultant nymphs exhibit five instars. Both sexes reach sexual maturity within 24 h after eclosion (UKL, unpublished data). Females live, on average, for 30 d and lay about 150 eggs. Generally, a sex ratio of 1:1 is maintained throughout the year and both sexes mate multiple times (UKL, unpubl. data).

Insect and Plant Collection and Maintenance Psyllids and host plant branchlets were collected from *A. verticillata* trees around La Trobe University (Melbourne, Vic., Australia). Insects were maintained in rearing containers and kept in a plant growth cabinet (Conviron Adaptis CMP 6010, Canada) maintained at 20 °C/10 °C and a 14:10 L:D photoperiod. Each rearing container consisted of a clear polycarbonate cylinder (6.5 cm diam. × 12 cm high, and sealed at one end with 1 × 1 mm mesh) and a plastic base (7.5 cm diam. × 6.5 cm high) containing moist floral foam with a circular opening at the center. Insects were provided with fresh branchlets every other day.

Analysis of Cuticular Hydrocarbons (CHCs) We collected

individuals as late instars on host branchlets or adults in teneral condition (when the cuticle was not fully sclerotized). Late instars were kept in rearing containers in the plant growth cabinet until eclosion. Following the teneral period, adults were separated by sex and kept in separate rearing containers according to age and sex. Twenty virgin male and female psyllids, 1, 2, 3, 7, and 14-d-old were randomly sampled from our collection. For each sex and age category, 50 % of the insects were allowed to mate. Insects in polypropylene vials (5 ml) were killed by freezing at −20 °C for 1 h. Individual insects then were inserted into separate 2 ml glass vials (Agilent Technologies, Australia Pty Ltd), air-dried in a desiccator for 72 h prior and shipped to Keele University (United Kingdom) for chemical analysis. Cuticular hydrocarbons were analyzed using the solid sampler technique (Bagnères and Morgan 1990). Samples were individually inserted in separate glass capillaries, and capillaries were sealed at both ends with a Bunsen burner. Each sample was heated inside the solid sampler in the injector port of a gas chromatograph at 285 °C for ~1 min and then crushed at the start of a run. Samples were analyzed in splitless mode. The column oven temperature was programmed from 70 °C (1 min hold) to 180 °C at 20 °C.min^{-1}, then to 320 °C at 10 ° C.min^{-1}, and held for 10 min. Helium was used as carrier gas at a constant flow of 1.0 ml.min^{-1}. All samples were analyzed using an HP 6890GC, equipped with a HP-5MS column (length 30 m, ID: 0.25 mm, film thickness: 0.25 μm; Agilent Technologies Inc., Santa Clara, CA, USA), coupled to an HP5973 Mass Selective Detector (electron impact ionization; 70 eV). Compounds were identified tentatively by key diagnostic ions, and Kovats' indices (Carlson et al. 1998). Alkanes were further identified using an alkane standard (C_{21}–C_{40}, Sigma Aldrich, St Louis, MO, USA). Our analyses of psyllid CHCs focused on compounds considered most likely to function as short-range sex attractants, *i.e.*, *n*-alkanes and methyl-branched alkanes (chain lengths of 21–37 carbons and with MW > 300), and which were most abundant or most consistently apparent in chromatograms. Based on these criteria, 25 peaks were examined for all the psyllids sampled (Fig. 2). Peak area was used to estimate the abundances and relative abundances of compounds.

Long-Range Y-tube Olfactometer Bioassay Male and female responses to odors of the opposite sex were tested in a 2.5 cm diam. Y-tube olfactometer, consisting of a 13 cm stem and two 8 cm arms, oriented at 110° to each other. Each arm was connected to an insect holding chamber.

Twenty five live psyllids of the same sex were added to the holding chamber of one of the arms while the other was left empty. Air entering each arm was purified through an activated charcoal filter, humidified, and the flow adjusted to 225 ml.min^{-1}. Air was drawn out of the system, at 450 ml min^{-1}, by a vacuum pump connected to the base of the Y-tube. Psyllids were collected in the afternoon, separated by sex, and allowed to feed on host branchlets overnight in separate rearing containers stored in the plant growth cabinet. Approximately 15 min. prior to bioassays, individuals were isolated in 5 ml polypropylene vials. An individual psyllid was introduced at the base of the olfactometer and allowed 10 min. to choose between the two arms before being removed. Individuals that did not make a choice within 10 min were discarded and not included in the analysis. Choice was defined when an individual contacted the end of an arm at the point of connection to the insect holding chamber. The study consisted of 10 replicates, comprising 12 individuals each. For each replicate, the arms of the olfactometer were rotated 180° horizontally after three individuals were assayed. After each replicate, the olfactometer was dismantled and cleaned in hot soapy water, followed by rinsing with acetone, and baking overnight at 220 °C.

Responses to Host Plant Branchlets Branchlets were collected from a single female tree. Eggs and nymphs were removed under a stereomicroscope and trimmed to 20 cm length. Branchlets were wiped gently with cotton wool soaked in hexane to remove putative chemical cues left by psyllids. Individual, cleaned branchlets were kept in separate rearing containers. Cleaned branchlets were exposed to psyllids by introducing either 10 males or 10 females into a rearing container and allowing them to feed overnight. The following morning, psyllids were removed from the branchlets, and pairs of branchlets were inserted into clean rearing containers for comparison of the following: (1) previously occupied by males *vs.* previously unoccupied, (2) previously occupied by females *vs.* previously unoccupied, or (3) previously occupied by males *vs.* previously occupied by females. Ten males and females were introduced into separate sets of rearing containers with various treatments. A longitudinal study design was conducted in which the number of psyllids that settled on each branchlet was scored every 30 min. from 10:00 am until 5:00 pm. Each time a score was taken, rearing containers were gently (to avoid disturbing psyllids) rotated through 180°. The bioassay was replicated ten times with males and nine times with females. Individual

psyllids were not re-used.

Short-Range Chemoreception Bioassay These bioassays were conducted in 15 cm diam. glass Petri dishes (Fig. 1). Males used in this bioassay were separated from females for 24 h before being assayed. Selected male and female psyllids were freeze killed an hour prior to commencement of the bioassay, and their CHCs were removed by 3 successive immersions, of 1 min each, in 300 µl of hexane with gentle agitation. By this method, we were able to reduce CHCs by up to 97 % (Fig. 2). Freshly killed psyllids (with intact CHCs *vs.* treated with hexane) were pinned through the thorax onto individual branchlets at the midpoint using micro pins, in separate Petri dishes. A single male was introduced into the arena at the extreme end of the branchlet. The residence time of males around freshly killed psyllids was recorded for 10 min, and courtship behavior, such as raising/extruding the adeagus and attempts to mate, were noted. *In copula* pairs were separated after 5 min.

Fig. 1

The design of the short-range chemo-reception bioassay

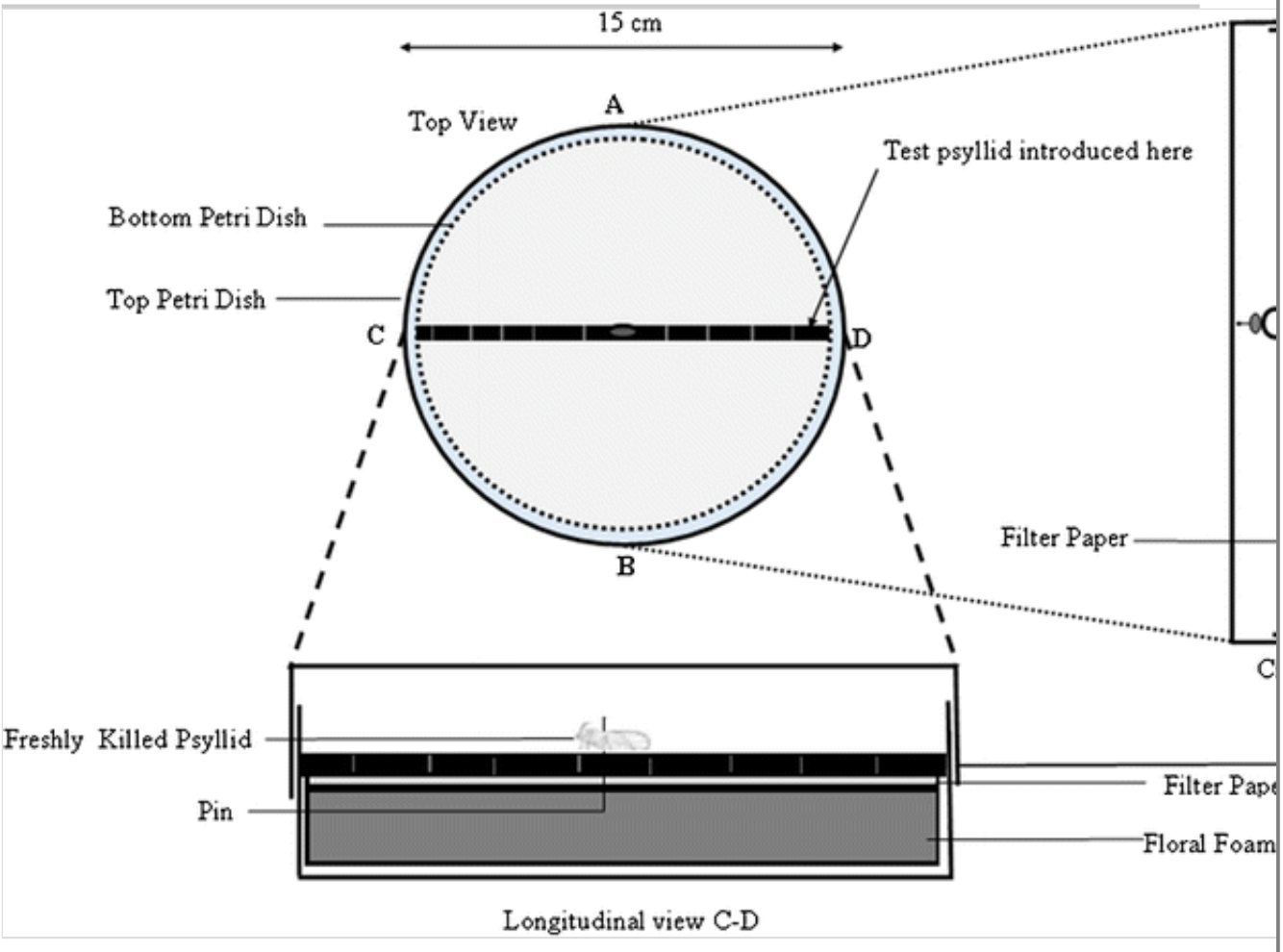
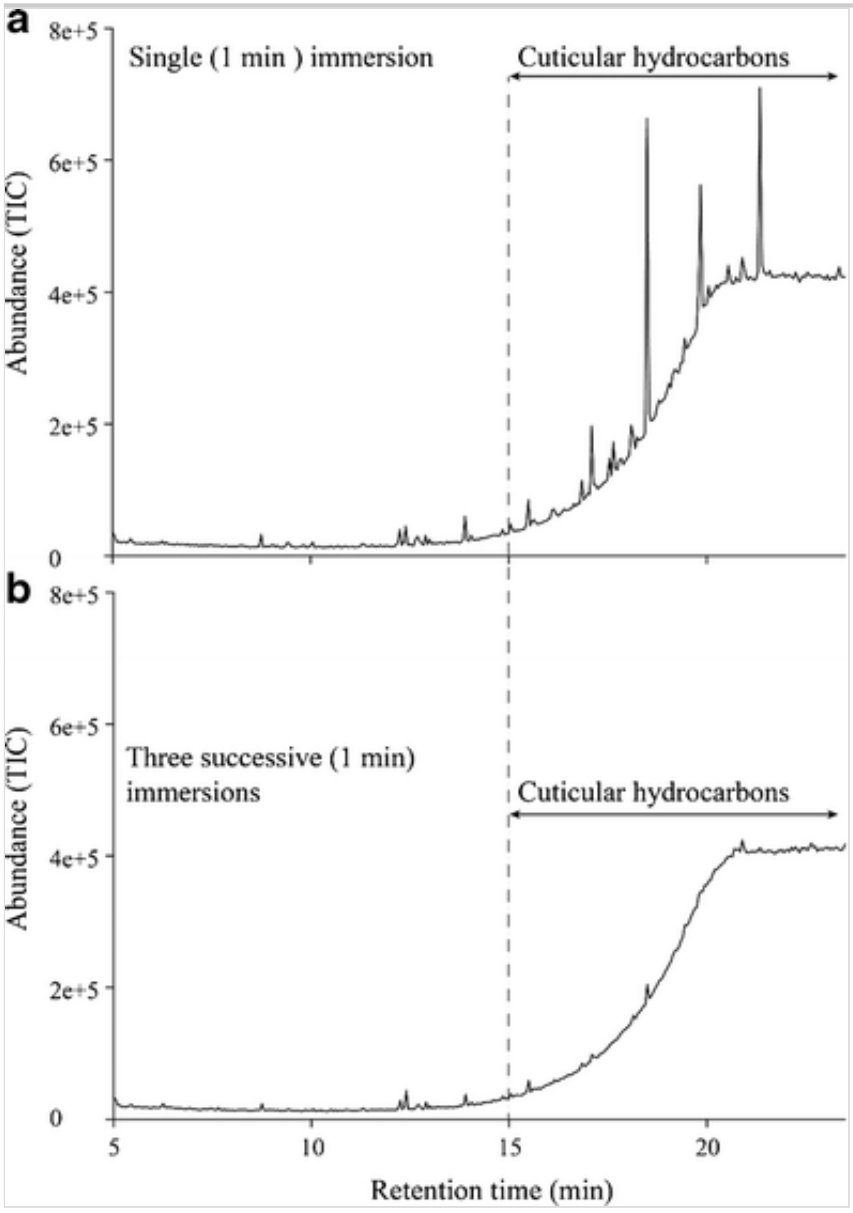


Fig. 2

Comparison of *Aacanthocnema dobsoni* cuticular body extracts after a single (1 min) immersion in 300 µl hexane (**a**) or three successive (1 min) immersions in 300 µl hexane each (**b**). Note, all major peaks were reduced substantially in **b**



Statistical Analyses A logit transformation was used to normalize CHC relative abundance data (Warton and Hui 2011). To consider the influence of the factors sex, age, and mating status, permutational multivariate analysis of variance (PERMANOVA) using the Bray-Curtis similarity matrix with 999 permutations was used (Anderson 2001). Analyses were conducted using PRIMER version 6.1.12. Univariate one-way analysis of variance (ANOVA) was used to compare the relative abundances of

individual peaks. Pearson's *Chi-square tests* (χ^2) were used to compare male and female responses in the long-range, Y-tube olfactometer bioassays. The preferences of males and females for previously occupied branchlets were compared using a generalized linear mixed model (glmerMod) of the binomial (logit) family and fitted using maximum likelihood (Laplace approximation) with treatment and time as fixed effects. This model enabled us to compare changes in psyllid settlement over time. This test was conducted using R-statistical software. Residence times of males around freshly killed conspecifics were compared using the Mann–Whitney *U* tests. *Chi-square* tests, Mann–Whitney *U* tests, and ANOVAs were performed using SPSS statistics 22.

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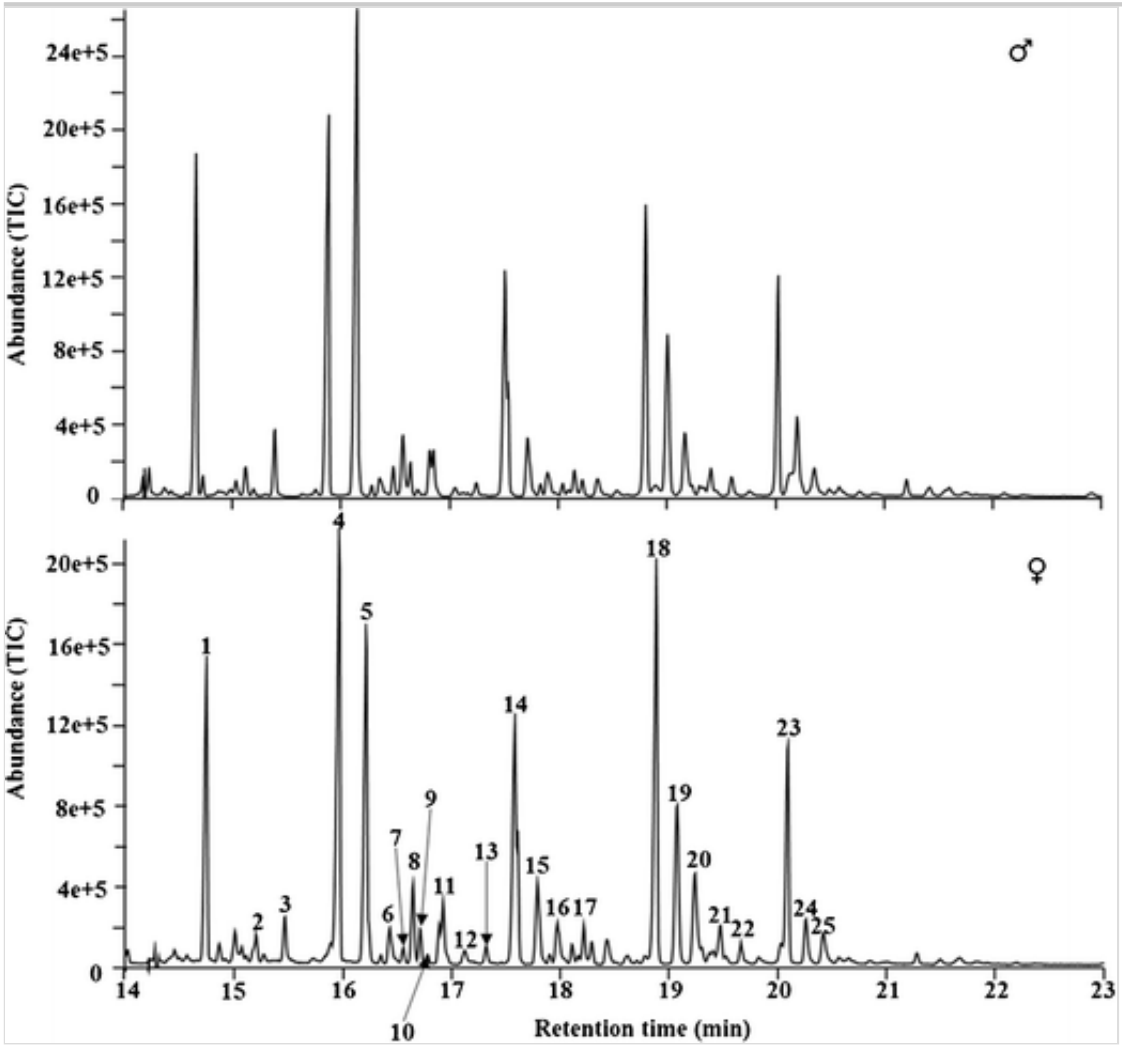
Results

Analysis of CHCs The 25 peaks selected comprised 34 CHCs, ranging from *n*-C_{25} to *n*-C_{33} (Fig. 3). These included nine *n*-alkanes, 17 monomethyl alkanes, and eight dimethyl alkanes (Supplementary Table 1). Two compounds co-eluted at each of the following times: 16.40 min (11- and 13-MeC_{27}), 17.75 min (11- and 13-MeC_{29}), 19.03 min (11- and 13-MeC_{31}), 19.19 min (11-,15-diMeC_{31} and 13-,17-diMeC_{31}), and 20.36 min (11-,15-diMeC_{33} and 13-,17-diMeC_{33}). Four compounds (11-, 13-, 15-, and 17-MeC_{33}) co-eluted at 20.21 min. Data for the 25 peaks are presented, but compounds that co-eluted are not discussed further since their individual abundances could not be resolved.

Fig. 3

Total ion mass chromatograms (TIC) of *Aacanthocnema dobsoni* cuticular hydrocarbon extracts of a single 1-d-old virgin male (*top*) or female (*bottom*) analyzed by gas chromatography/mass spectrometry (GC/MS) using a solid sampler technique. Peak identities: 1 = *n*-C_{25}, 2 = 2-MeC_{25}, 3 = *n*-C_{26}, 4 = 2-MeC_{26}, 5 = *n*-C_{27}, 6 = 11- & 13-MeC_{27}, 7 = 5-MeC_{27}, 8 = 2-MeC_{27}, 9 = 3-MeC_{27}, 10 = 5-,15-diMeC_{27}, 11 = *n*-C_{28}, 12 = 3-,7-diMeC_{27}, 13 = 2-MeC_{28}, 14 = *n*-C_{29}, 15 = 11- & 13-MeC_{29}, 16 = 11-,15-diMeC_{29}, 17 = *n*-C_{30}, 18 = *n*-C_{31}, 19 = 11- & 13-MeC_{31}, 20 = 11-,15-diMeC_{31} & 13-,17-diMeC_{31}, 21 = *n*-C_{32}, 22 = MeC_{32}, 23 = *n*-C_{33}, 24 = 11-, 13-, 15- & 17-MeC_{33}, 25 = 11-,15-diMeC_{33} & 13-,17-diMeC_{33}. Relative abundances are given in Supplementary Tables 1 (both sexes), 2 (males) and 3 (females)



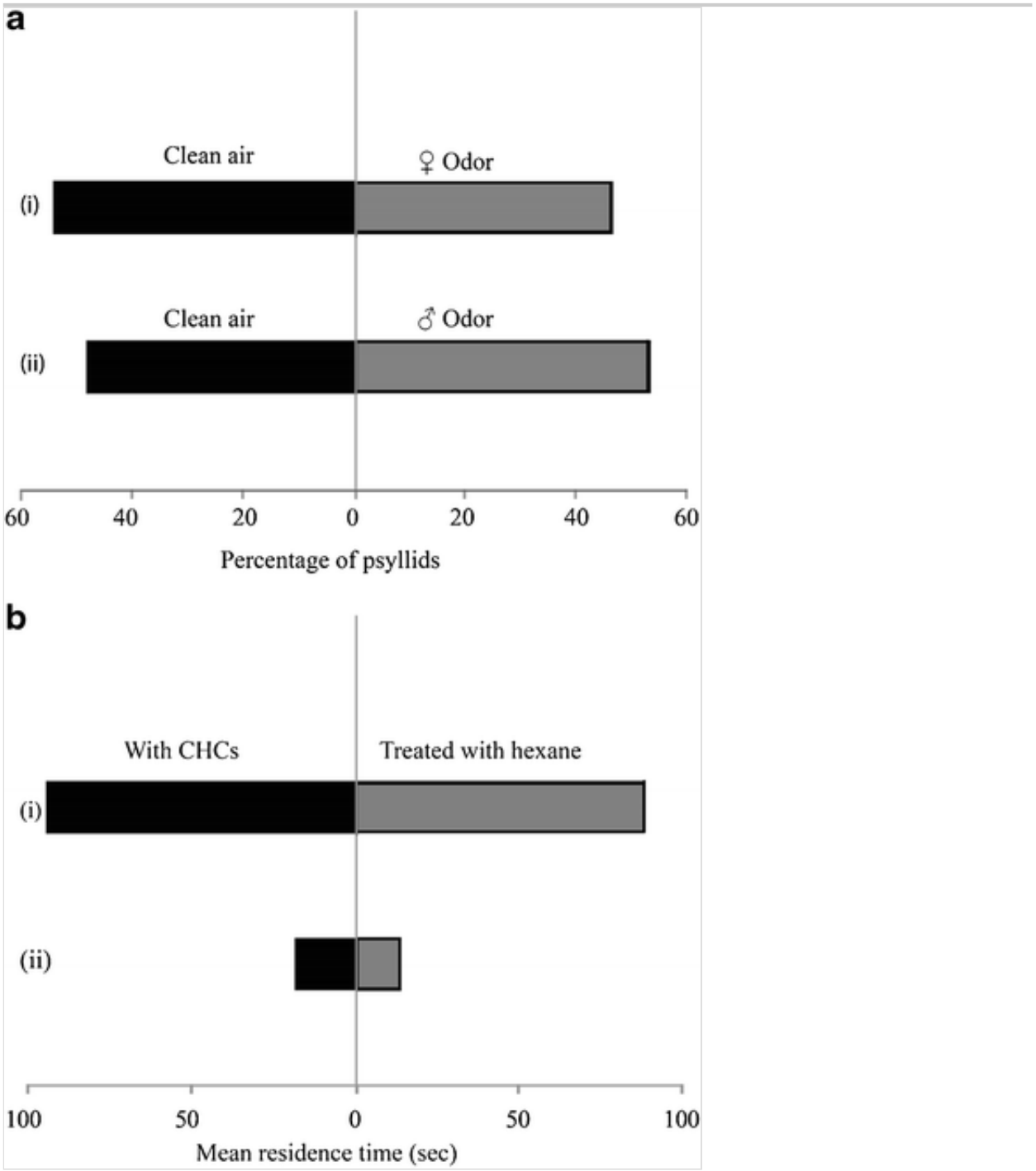
Cuticular hydrocarbon profiles of both sexes were dominated by *n*-alkanes (*n*-$C_{31,}$ *n*-C_{25}, *n*-C_{27}, *n*-C_{29}, *n*-C_{33}) and a mono-methyl alkane (2-MeC_{26}). Profiles varied between sexes (PERMANOVA; $df = 1$, $F = 13.9$, $P < 0.001$) (Fig. 3). Males had higher relative abundances of 2-MeC_{28}, 11, 15-di MeC_{29}, and *n*-C_{33}, whereas females had higher relative abundances of 5-MeC_{27}, 3-MeC_{27}; 5, 15-di MeC_{27}; *n*-C_{29} and *n*-C_{30} (Supplementary Table 1). Relative abundances of CHCs also varied among age groups of the same sex (PERMANOVA; $df = 4$, $F = 19.6$, $P < 0.001$). In males, 14 CHCs varied with age. Of these, four increased, eight decreased, while two fluctuated without showing any obvious trend (Supplementary Table 2). In females, 13 CHCs varied with age; six increased, three decreased, and four fluctuated without showing any obvious trend (Supplementary Table 3).

Long-Range Sex Attractants Males did not show any preference when provided a choice between clean air and female odors ($\chi^2 = 0.30$, *d.f.* =1, $P = 0.58$; Fig. 4Ai). Similarly, females showed no preference when offered a

choice between clean air and male odors ($\chi^2 = 0.83$, *d.f.* =1, $P = 0.36$; Fig. 4Aii).

Fig. 4

Summary of results from behavioral bioassays with adult *Aacanthocnema dobsoni*. **a** Responses of males (*i*) and females (*ii*) to odors emanating from groups of conspecifics ($N = 25$) of the opposite sex *versus* clean air in a Y-tube olfactometer. **b** Residence times (time spent around freshly killed conspecifics) of males around females (*i*) and males (*ii*) with intact cuticular hydrocarbon profiles *versus* individuals treated with hexane in a short-range chemo-reception bioassay. There were no differences ($P > 0.05$) between treatments in any of the comparisons

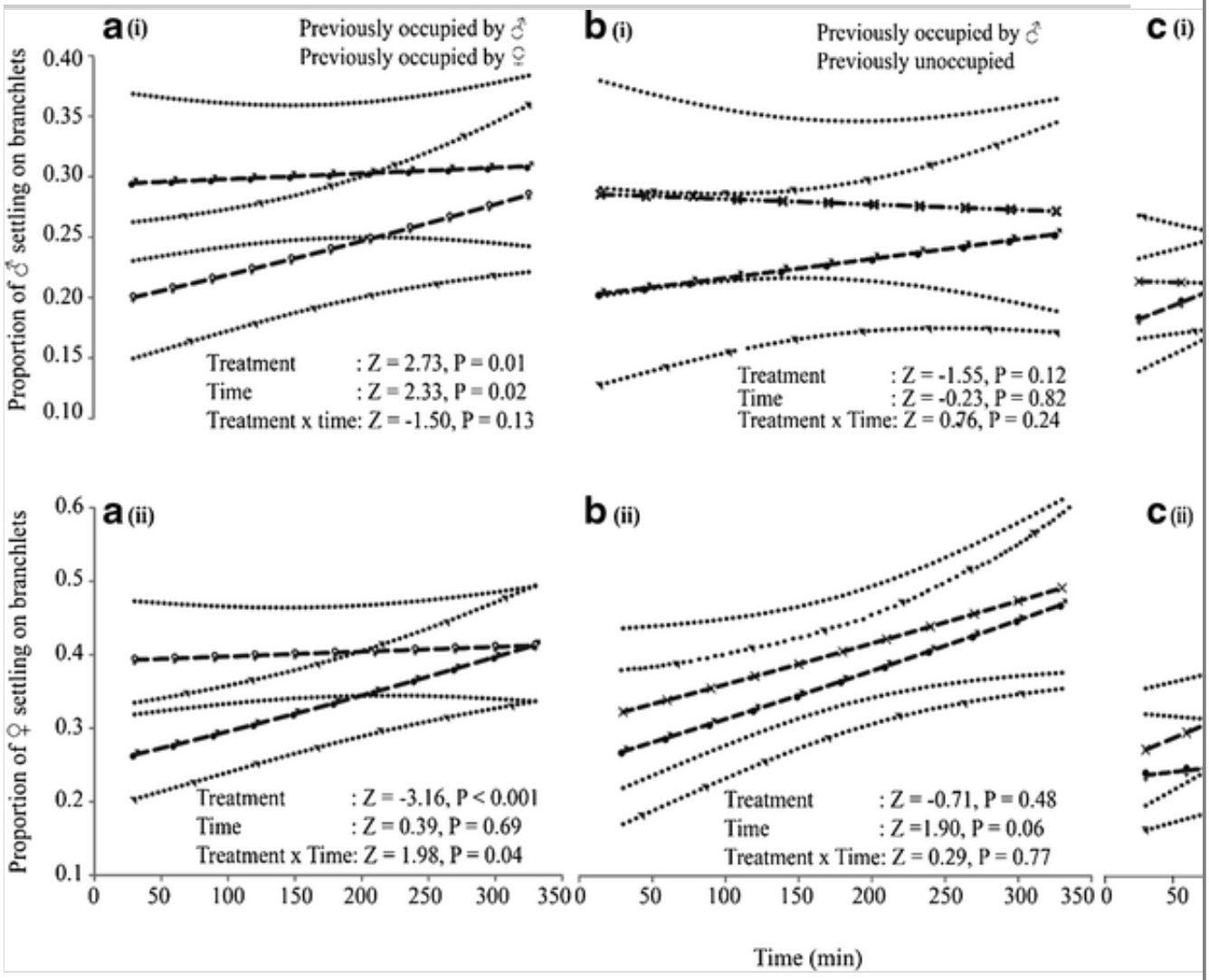


Short-Range Chemoreception Bioassay The residence time of males around freshly killed females with intact CHCs *vs.* freshly killed females treated with hexane did not differ ($U = 751$, $N = 39$ and 42, $P = 0.52$; Fig. 4Bi). Courtship behavior was exhibited in 52 % of instances in which males made contact with freshly killed females with intact CHCs and 46 % when males came into contact with freshly killed females treated with hexane. Males copulated in 18 % or 15 % of instances when they were presented with freshly killed females with intact CHCs or treated with hexane, respectively. Similarly, there was no difference in male residence time around freshly killed males with intact CHCs and around freshly killed males treated with hexane ($U = 708$, $N = 42$ and 35, $P = 0.780$; Fig. 4Bii). Courtship behavior was exhibited in 32 % of instances in which males made contact with freshly killed males with intact CHCs and in 20 % of instances when males came into contact with freshly killed males that were treated with hexane. Although males attempted to mate with freshly killed males, copulation was never successful.

Responses to Host Plant Branchlets When offered a choice between branchlets, previously occupied by males *vs.* those previously occupied by females, males initially (within the first 30 min) preferred to settle on the former. However, male settling was affected by time. As the assay progressed, rates for male settling gradually became similar between branchlets (Fig. 5Ai). There was no interaction between treatment and time. Males did not show any obvious preferences for branchlets in the other two tests: previously occupied by males *vs.* previously unoccupied (Fig. 5Bi) and previously occupied by females *vs.* previously unoccupied (Fig. 5Ci). When females were offered a choice between branchlets previously occupied by males and those previously occupied by females, settling was affected by the interaction between treatment and time. Thirty minutes post commencement of the bioassay, female settling was higher on branchlets that had been previously occupied by females. Over time, female settlement on the branchlet previously occupied by males gradually increased and matched settlement on branchlets previously occupied by females (Fig. 5Aii). Female preferences did not differ between branchlets in any of the other tests: previously occupied by males *vs.* previously unoccupied (Fig. 4Bii) and previously occupied by females *vs.* previously unoccupied (Fig 4ii).

Fig. 5

Responses of *Aacanthocnema dobsoni* males [**a**–**c**(*i*)] and females [**a**–**c** (*ii*)] to host plant branchlets previously occupied by conspecifics *versus* branches that had not been occupied previously. *Dotted lines* = 95 % confidence intervals



Discussion

Insect CHCs are complex traits with ancient origins that have been shaped by environmental and endogenous factors (Leonhardt et al. 2013; Martin et al. 2008a). Despite growing evidence that these compounds provide essential protection against desiccation and are used for communication in taxa such as Hymenoptera, Coleoptera, and Lepidoptera (Guerrieri et al. 2009; Heuskin et al. 2014; Zhang et al. 2014), little is known about the nature and biological function of CHCs in Psylloidea. We have shown that the CHC profile of *A. dobsoni* is dominated by *n*-alkanes, monomethyl-,

and dimethyl alkanes. These compounds were present in quantities that varied according to sex and age. While sex-linked differences appear to be important for mate attraction in *C. pyricola* and *D. citri* (Guédot et al. 2009b; Mann et al. 2012), we found no similar evidence for *A. dobsoni*. We suggest that age-related differences in the CHCs of *A. dobsoni* probably reflect losses through abrasion given that the molecular weights and boiling points of most of the CHCs are probably too high for them to volatilize to any significant extent.

Cuticular Hydrocarbon Profile Although the CHCs of *A. dobsoni* have not been previously analyzed chemically, they consist of compounds that have been identified in several other insect taxa, reflecting a high degree of conservatism (Guédot et al. 2009b; Martin et al. 2008a; Martin and Drijfhout 2009). Sixty two percent of the CHCs we identified are also known on *C. pyricola* (Guédot et al. 2009b). Most of the common compounds are *n*-alkanes and monomethyl alkanes, but the same dimethyl alkanes are not common to both. The common compounds most likely are utilized in different ways by these species. For example, 13-MeC_{27} is present in both species, and is known to function as a sex attractant in *C. pyricola* (Guédot et al. 2009b) but not in *A. dobsoni*. The lack of shared dimethyl alkanes between *A. dobsoni* and *C. pyricola* is not surprising because these compounds are, in most cases, species-specific and often are utilized for species recognition in higher insect orders such as Hymenoptera (Guerrieri et al. 2009; Martin et al. 2008b). The majority of the *n*-alkanes and monomethyl alkanes likely provide protection against water loss. It is unlikely that *n*-alkanes, and, to some extent, mono-methyl alkanes, function as stable and distinctive communication signals (Gibbs and Pomonis 1995; Howard and Blomquist 2005; Martin and Drijfhout 2009) because these compounds are shared across taxa and vary considerably depending on environmental factors (Gibbs and Pomonis 1995; Gibbs 1998).

Sex- and Age-Specificity We found that the CHC profiles of males and females differ with respect to quantities of specific compounds: 2-MeC_{28}, 11,15-diMeC_{29}, and *n*-C_{33} (produced in higher proportions in males) and 5-MeC_{27}, 3-MeC_{27}, 5,15-diMeC_{27}, *n*-C_{29}, and *n*-C_{30} (produced in higher proportions in females) (Supplementary Table 1). Sex-related differences in CHC profiles also have been reported in *C. pyricola* and *D. citri* (Guédot et al. 2009b; Mann et al. 2012). Post-diapause *C. pyricola*

females produce higher quantities of 13-MeC_{27} compared to post-diapause males (Guédot et al. 2009b), while *D. citri* females produce more $C_{12}H_{24}O_2$ compared to conspecific males (Mann et al. 2012). We also found that the CHC profiles of both sexes vary with age. Although not previously reported in Psylloidea (Sternorrhyncha), age-related differences in CHC profiles have been reported in Heteroptera, *e.g.*, the Bean bug (*Riptortus pedestris*) (Alydidae) (Yoon et al. 2012). In Psylloidea, the use of sex-related differences in CHC profiles for mate attraction has received considerable attention (Guédot et al. 2009b; Mann et al. 2012). However, age–related differences in CHC profiles as indicators of mate quality have been ignored. Age–related differences in CHCs may occur simply through abrasion (Dirks and Taylor 2012). Nevertheless, such differences are known to provide vital clues to choosy females about mate quality, especially in species such as *Ephippiger ephippiger* (Orthoptera: Tettigoniidae), in which mate quality varies with age (Wedell and Ritchie 2004). In Psylloidea, males of the Citrus psylla, *Trioza erytreae* Del Guercio (Triozidae), provide a good example of male mate quality varying with age. *Trioza erytreae* males mate multiple times, although their ability to fertilize females decreases with age (Van den Berg et al. 1991). Therefore, females of *T. erytreae* could utilize age-related differences in CHC profiles to assess and choose between younger and older males, which would be of benefit if mating with the former were likely to result in increased fitness.

Long-Range Activity of CHCs Males of some psyllid species, such as *C. pyricola, C. bidens*, *B. cockerelli*, and *D. citri*, are attracted to conspecific female odors (Guédot et al. 2009b, 2010; Horton et al. 2008; Wenninger et al. 2008). However, we obtained no evidence that *A. dobsoni* utilizes CHCs for long-range mate attraction. This was not surprising given that most of the compounds we identified have molecular weights above the putative upper limit (MW of 300) for airborne sex pheromones (Bradbury and Vehrencamp 2011b). Surprisingly, 13-MeC_{27}, a compound identified as a long-range sex attractant in *C. pyricola*, has a MW of 394 and a boiling point of 466.3 ± 12.0 °C at 760 mm Hg (Pence and Williams 2010) and is, therefore, expected to have only short-range activity. Insect taxa such as Lepidoptera and Hymenoptera that rely on volatile (low MW) chemical compounds for long-range mate attraction have well developed pheromone glands responsible for the production of such compounds (Niño et al. 2013). However, we are not aware of any

study that has demonstrated the presence of pheromone glands in a psylloid.

Short-Range Activity of CHCs We also found no evidence that either males or females leave chemical cues on host plants that might influence the behavior of conspecifics of the opposite sex. Our results contradict findings by Brown et al. (2009) who showed that males of *C. pyricola* were more attracted to pear shoots previously occupied by females compared to shoots previously unoccupied or occupied by males. Nevertheless, during the first 30 min. of our bioassay, males and females appeared to be weakly attracted to branchlets previously occupied by conspecifics of the same sex (Fig. 5Ai & ii). The initial attraction of *A. dobsoni* females to branchlets previously occupied by females could have been due to attraction to conspecific eggs. Although branchlets were initially cleaned of eggs, cleaned branchlets that were exposed to female psyllids had eggs oviposited on them prior to their use in the bioassay. Many insects are known to be attracted to conspecific eggs; their presence is thought to indicate to other females that those locations are high-quality resources (*e.g.*, Raitanen et al. 2013; Stephan et al. 2015). However, as the density of conspecific females increased, intraspecific competition may have intensified and reduced the attractiveness of branchlets (Raitanen et al. 2013). This could explain why, over time, female settling on branchlets previously occupied by males gradually increased and matched settling on branchlets previously occupied by females.

We also found no evidence that the CHCs are utilized for sex recognition by *A. dobsoni* males. Interestingly, males attempted to copulate with freshly killed males as readily as they did with freshly killed females. We only used males in our short-range chemoreception bioassay; therefore, we do not know whether differences in male CHC profiles may be utilized by females to recognize and possibly choose among males. Females of some psyllid species, such as *Trioza erytreae* (Hemiptera: Triozidae), *C. densitexta,*, and *C. pyricola*, have been reported to exhibit selectivity for individual males (Krysan 1990; Van den Berg et al. 1991). Although we found no evidence that *A. dobsoni* males utilize CHCs to recognize females, some of the compounds we found play critical roles in mediating behavior in other insect taxa. For example, in *Lariophagus distinguendus* (Hymenoptera: Pteromalidae), 3-MeC_{27} is a key component of a contact sex pheromone that triggers courtship behavior in males (Kühbandner et al.

2012), while 2-MeC_{28} is a component in a blend of compounds utilized by fifth instars of the codling moth, *Cydia pomonella* (Lepidoptera: Olethreutidae), as an aggregation pheromone (Jumean et al. 2005, 2007).

In summary, while *A. dobsoni* is known to utilize SBVs for communication (Percy et al. 2006), we obtained no evidence that it uses semiochemicals for either long-range mate attraction or short-range mate recognition, in contrast to the situation in a number of psyllid species from the northern hemisphere. Some psyllid species, such as *D. citri* and *C. pyri*, utilize a multimodal signaling system involving a combination of both SBVs and semiochemicals (Eben et al. 2014; Mankin et al. 2013; Wenninger et al. 2009). So, why are semiochemicals apparently an unimportant component of the signaling repertoire of *A. dobsoni*? It is probable that the signaling behavior of *A. dobsoni* has been shaped strongly by the physical characteristics of its host's branchlets. *Allocasuarina verticillata* is characterized by cylindrical (0.7–1.5 mm diam) (Wilson and Johnson 1989) branchlets, which can be up to 80 cm long (UKL, unpubl. data). Substrate-borne vibrations travel through plant materials with low attenuation, and can enable long-range communication of up to 2 m on the same plant (Čokl and Virant-Doberlet 2003). Consequently, the branchlets of *A. verticillata* likely are highly suitable for long-range communication, especially compared to the situation faced by species of psyllids that live on flat leaves. Future work should concentrate on how SBVs are used for communication by *A. dobsoni* and how a host's biophysical properties influence transmission of these signals. Together, this information will help us understand how the biophysical characteristics of this psyllid's host plant has shifted the form of communication used by *A. dobsoni*, seemingly to the detriment of semiochemical communication.

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Electronic supplementary material

Below is the link to the electronic supplementary material.

ESM 1

(DOCX 24 kb)

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